Time evolution of Pb(II) speciation in Pampa soil fractions

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ABSTRACT

Soil pollution by heavy metals, particularly lead, is an important environmental concern; the bioavailability of such pollutants is strongly dependent on their chemical form. Here, the speciation of Pb(II) in soil fractions as a function of time shortly after its incorporation is studied, using a selective sequential extraction method. The sample came from an Argentinean Pampas region and was extensively characterised, including Rietveld analysis of the silt+clay fraction XRD pattern to find the major mineral components. Experiments were run twice, once in the winter and once in the summer. The results show different speciation time profiles in both cases, showing faster changes in winter due to the higher water content. The summer experiment corresponds to an earlier stage in the speciation profile evolution compared with winter. The soluble/ exchangeable fraction decreases with time in summer but shows a lower and constant value in the winter. A high proportion is found to be adsorbed onto the stable (aluminosilicates+quartz) mineral fraction. The results strongly suggest that, even at a short time following soil pollution with Pb, a high proportion is adsorbed onto the mineral fraction, with only a low fraction being bioavailable. The most stable (mineral incorporated) form is observed to increase with time. Soil water content appears to be more important than temperature in determining the differences between the two.

Keywords: sequential selective extraction, bioavailability of pollutants, heavy metals

INTRODUCTION

Lead is a well known pollutant, highly toxic to humans (Needleman, 2004), which can be found in the environment due to a large variety of anthropic activities, such as mining and industrial activities (Arrouays *et al.,* 1996), painting and enamels (Needleman, 2004), lead pipes (Cartier *et al.,* 2013), lead shot hunting (Ferreyra *et al.*, 2009; Selonen *et al.*, 2012), car battery residues (Hamzeh *et al.*, 2011) and fuel additives, not used currently but remaining in the environment from past uses (Farmer *et al.*, 1997; Lavado *et al.,* 1998; Mielke *et al.*, 2011). From the soil, this pollutant, usually a Pb(II) soluble species, can be absorbed by plants, reaching even the grains and be transmitted to animals and humans (Lavado *et al.*, 2007). However, its bioavailability is dependent on the Pb speciation in soil (Shahid *et al.*, 2012), it has been observed that adsorbed (*i.e.* bound to soil colloids) Pb is not bioavailable as determined by bacterial reporters (Magrisso *et al.*, 2009).

Thus, determination of the speciation of pollutants, such as lead, in soils is more relevant than a simple measurement of total metal contents. Sequential selective extraction (SSE) methods have been proposed to assess speciation of sorbed species between different soil fractions (Tessier *et al.*, 1979; McGrath and Cegarra, 1992; Ma and Uren, 1998; Hass and Fine, 2010). The SSE methods are based on the exposure of a sample to a sequence of wet chemistry treatments. At each step of the sequence the sample is extracted in a solution that either selectively removes or dissolves a specific solid component and assumes that the sorbed species are associated with or desorbs a specific adsorbate fraction, the extractants being increasingly strong in each step. Several

procedures have been proposed for determining the speciation of metals (Gleyzes *et al.*, 2002; Rao *et al.*, 2008). Some shortcomings have been distinguished: limited selectivity of the extractants, redistribution of elements among phases during extraction and limited comparability of data obtained by several investigators. Thus, in general, the accuracy of these procedures could be hampered due to overlay between fractions; nevertheless, they are considered to be useful and are actively applied in different soils and conditions (Rao *et al.,* 2008; Torri and Lavado, 2008; Orroño and Lavado, 2009; Kabala *et al.*, 2011; Garrido and Helmhart, 2012; Singani and Ahmadi, 2012). The successive steps are often assumed to remove the pollutant associated with a given soil component or fraction; however, due to the lack of complete knowledge of the soil composition, it is more appropriate to relate the fractions to the reactants employed giving an operational definition (Rao *et al.,* 2008; Kabala *et al.,* 2011).

Among other pollutants, Pb speciation in different soils was studied by several authors through SSE procedures (Jensen *et al.*, 2006; Ndzangou *et al.*, 2006; Kaplan and Yaman, 2009; Yang *et al.,* 2010; Malandrino *et al.,* 2011). Most studies focused on the origin of the Pb source and its properties, but not on the influence of climate conditions on soils at the time of pollution. To the best of our knowledge, there are no previous reports on this aspect. Climate conditions can influence the metal speciation by, for example, altering the rate of evolution towards stable forms and ultimately affecting bioavailability. Thus, the objective of this work is to study the influence of climate on the speciation of Pb(II) in soil fractions as a function of time, shortly after its incorporation into the soil. The speciation is studied experimentally using a SSE procedure following Ma and Uren (1998) with some modifications giving an operational definition which can be better correlated with bioavailability or functional definition. The experiment was run twice, once corresponding to winter conditions and the other to summer conditions.

MATERIALS AND METHODS

Soil sample characterisation

Soil sample

The studied soil was Typic Argiudoll (USDA Soil Taxonomy) from the Solis area in Buenos Aires province, Argentina (34º 18' S, 59º 20' W). The area is devoted to field crops and cattle husbandry, and recognised as a non polluted region (Lavado *et al.,* 2004). In a 1000 m^2 plot, 20 soil subsamples were taken at 0–0.20 m, mixed manually thoroughly. From this homogenised soil mass, the sample employed used in present research was obtained. The soil was air dried and passed through a 2 mm stainless steel sieve. The coarse sand, fine sand and silt + clay fractions were separated by appropriate sieving.

Chemical characterisation

All chemicals employed were analytical grade; ultrapure water from a Millipore MilliQ system was employed throughout. The pH was measured following (Thomas, 1996) in water, 0.01 M $CaCl_2$ and 1 M KCl. Cation exchange capacity (CEC) was measured by the compulsive exchange method (Sumner and Miller, 1996). Soluble anions were determined by ionic chromatography, after soaking 1.00 g of soil in 10 mL of water, with continuous shaking, for 1 hour; the chromatographic analysis was performed with a DIONEX AS4A column with 1.8 mM Na_2CO_3 – 1.7 mM $NaHCO_3$ at 2.0 mL min^{-1}. The mineral soil fraction was obtained after soil organic matter (SOM) destruction with 30% H_2O_2 in acetate/acetic acid buffer; SOM contents was determined by weight measurements obtained before and after its destruction. The heavy metals contents was found by atomic absorption spectrometry (AAS) with air/acetylene flame after acid digestion (Wright and Stuczynski, 1996) using a Shimadzu AA 6800. The final residue of extraction was completely mineralised by microwave digestion in PTFE closed vessels (CEM, MDS 2000), running the program recommended by the manufacturer.

X-ray diffraction

X-Ray powder diffraction analysis was performed with a Philips X-Pert diffractometer using CuK_α radiation (λ = 0.154056 nm). Diffractograms of the whole soil were not useful for analysis due to the presence of soil organic matter. The DRX pattern of the silt-clay fraction was obtained and analysed with the help of the MAUD program using the Rietveld refinement method (Lutterotti *et al.,* 1999).

Microscopic observation

To analyse the mineral composition, the soil mineral fractions (whole soil and silt + clay fraction) were examined under an optical microscope. Further observation was performed by scanning electron microscopy (SEM), using a Zeiss Supra 40 Gemini microscope. The samples were prepared by placing a small amount of solid on one side of a carbon tape.

Infrared spectroscopy

IR spectroscopy was performed for control and contaminated samples using a Fourier transform IR (FTIR) Nicolet 8700 spectrometer, in the range 400–4000 cm^{-1}. The samples were pressed into pellets prepared dispersing 0.5 mg of each one in 150 mg of KBr. For each sample, 32 scans were accumulated and averaged.

Pb speciation experiments

Two experiments on Pb sorption and speciation in soil were conducted, the first one in August/September under cold conditions (typical of winter in this region of the Southern Hemisphere) and the second in summer (mid-December to mid-February) having warm climate. Pots of 2 L capacity

were loaded with 2 kg soil each and a $Pb(NO_3)_2$ solution of the appropriate concentration as to obtain final doses of 200, 2000 or 10000 mg Pb per kg of soil, was added. The pots were placed in a shelter and irrigated at 7 day intervals at 70% field capacity; samples were collected at specified times during the experimental period of 60 days. The 200 mg kg^{-1} total Pb samples were analysed by the SSE procedure detailed above, because this level corresponds to typical values on polluted soils. More concentrated samples were employed for IR spectra recording. Duplicate or triplicate runs were performed in most cases; the repeatability was verified in a quintuplicate experiment and found to be satisfactory (the standard error was below 3% for all fractions).

Sequential selective extraction procedure

In an SSE, the different steps after the first are expected to selectively dissolve/destroy a particular solid (or groups of solids) fraction, with reactants of increasing strength, releasing the associated metal ions. It is in principle assumed that the metal is mainly incorporated into the solid, however, surface adsorbed species should also be removed in the course of the procedure. One of such methods is the Ma and Uren procedure (Ma and Uren, 1998); this method was modified for the purposes of the present work; the procedure employed is shown in Table 1, where the steps are operationally defined; the procedure is discussed in the following:

1. A 1 M $MgCl_2$ solution is used in step I (Table 1) to remove soluble and exchangeable Pb. The use of a chloride salt could cause the precipitation of low solubility salts such as $PbCl_2$ and PbClOH, however, the Cl$^-$ ion also forms complex ions $PbCl_3^-$ and $PbCl_4^{2-}$ through the following reactions:

$$Pb^{2+}(aq) + Cl^-(aq) \rightleftharpoons PbCl^+(aq) \tag{1}$$

$$PbCl^+(aq) + Cl^-(aq) \rightleftharpoons PbCl_2(aq) \tag{2}$$

$$PbCl_2(aq) + Cl^-(aq) \rightleftharpoons PbCl_3^-(aq) \tag{3}$$

$$PbCl_3^-(aq) + Cl^-(aq) \rightleftharpoons PbCl_4^{2-}(aq) \tag{4}$$

The precipitation of lead chloride can be simply written as:

$$PbCl_2(aq) \rightleftharpoons PbCl_2(s) \tag{5}$$

Another solid phase which can appear is basic lead chloride as:

$$PbCl^+(aq) + OH^-(aq) \rightleftharpoons PbClOH(s) \tag{6}$$

An estimation of the Pb(II) speciation according to reactions (1)–(6) [other possible species, such as Pb–OH complexes and $Pb(OH)_2(s)$, are negligible in the experimental conditions] with the aid of the Visual Minteq program (Gustafsson, 2011) was performed. Due to the high ionic strength (> 1 M), the computation of activity coefficients should be consideredwith caution. Here, two methods were employed: the first uses the specific ion interaction theory

Table 1 Sequential selective extraction procedure employed

Step	Fraction description	Extractant
I	Water soluble/exchangeable	$MgCl_2$ 1.0 M, shaken 1 hour
II	Associated to reducible components (*e.g.* Mn oxides).	Hidroquinone 0.2% / 1.0 M CH_3COONH_4 Shaken 1 hour
III	Bound to oxidisable components (mainly organic matter)	30% H_2O_2 (5 mL portions until end of reaction) in 0.5 M $NaCH_3COO/CH_3COOH$ at 80 ºC Shaken 1 more hour
IV	Oxalate extractable (*e.g.* Fe/ Al oxides or adsorbed to other minerals)	0.175 M $(NH_4)_2C_2O_4$ / 0.1 M $H_2C_2O_4$, standing for 15 hours and shaken for 2 more hours
V	Residue (mainly silicates)	Microwave digestion in H_2SO_4 (conc.) + $HClO_4$ (conc.) + HNO_3 (conc.) + HF (conc.)

(SIT) of Bronsted, Guggenheim and Scatchard (Scatchard, 1936), which takes into account specific interactions between ions in concentrated solutions; the parameters employed are those found in IUPAC and NEA, plus empirical estimations in those cases where experimental vales where not available (see Grenthe *et al.,* 1997 for details); the results indicate that in the experimental conditions (pH about 7.0) saturation $PbCl_2$ is reached at a total Pb concentration, c_{Pb}, of about 7.5 mM, whereas for PbClOH it is near 1.5 mM. Another estimation was conducted using the Davies equation (Davies, 1962) that is known to overestimate the activity coefficients at high ionic strengths which, in turn, leads to an underestimation of the saturation concentration; here saturation for $PbCl_2$ was given at c_{Pb} ~ 3 mM and for PbClOH at c_{Pb} ~ 1 mM. For a 200 ppm of Pb in soil experiment (which is of the same order of magnitude of typical polluted soils). If all metal were dissolved in the first step, c_{Pb} would be about 10^{-4} M, well below the above estimations.

2. In step II, the reactant is hydroquinone, a reductant which will act upon oxidant minerals such as manganese oxides promoting their dissolution and the consequent release of metal species, either incorporated into the solid or adsorbed onto it.

3. Step III it is an oxidative attack, which will destroy the organic fraction (other oxidisable materials are expected to be negligible) releasing bound metal cations.

4. Step IV employs the strongly complexant oxalate buffer; this step is commonly described as associated to iron and aluminium oxides.

5. Finally, a strong acid attack on the residue is performed as step V, in order to dissolve the Pb(II) present in its more resilient forms.

For the SSE procedure, the starting soil amount was 1.00 g. In each step, the soil was suspended in 10.0 mL of the corresponding extractant; the Pb contents of the respective fractions were determined by flame atomic absorption spectrometry.

RESULTS AND DISCUSSION

Soil characterisation

General and chemical characterisation

Table 2 presents the results of the general characterisation essays. It is observed that it is a moderately acid soil using several methodologies, predominating silt plus clay, with an important organic matter contents. The cation exchange capacity is low, which indicates low clay content, in agreement with the known top soil texture of the area (silty loam). Nitrates are predominant among the anions. A low concentration of Mn is found indicating relatively low contents of Mn oxides. Also, low concentrations of other heavy metals are observed; the Fe content is relatively small. The pristine soil is free of Pb within experimental error.

X-ray diffraction pattern

Figure 1 shows the XRD pattern of the silt+clay fraction; because the smaller particles are more important for ion binding, we will concentrate on that fraction. Some diffraction peaks are prominent, mainly that due to quartz at $2\theta = 26.62°$. The refinement using the Rita-Rista methodology (Ferrari and Lutterotti, 1994; Wenk *et al.,* 1994) is consistent with the presence of quartz (43%), low T albite (7%), high T albite (26%), orthoclase (14%) and small quantities of anhydrite, alunogen, biotite and haematite. Also, no evidence of carbonate minerals was found which is consistent with the relatively low pH values found. Additionally, following the established technique to distinguish phyllosilicate species (Amonette, 2002; Whittig and Allardice, 2010), the mineral fraction was subjected to homoionisation (exchanging all cations by a single species) first with Mg(II) and following with K(I), in the last case heated to 550 ºC, recording the DRX pattern in each step; no clear evidence of phyllosilicates was observed, thus these minerals, if any, would be present in a very small proportion.

Microscopical observation

Figure 2 shows typical SEM images of the soil sample; quartz and plagioclase minerals are observed, partially covered with organic matter, also some amorphous material is found. Optical microscopy shows mainly sedimentary clasts in the sand fractions, whereas in the finest fraction quartz, feldspars and some opaque minerals are observed; a small amount of amorphous material was also observed.

IR spectra

Figure 3 shows the IR spectra, which reveals interactions between Pb(II) and carboxylate groups. The band at ~1384 cm^{-1} (see inset) shows a sharply increase which has been reported to appear when heavy metals such as Hg(II) or

Table 2 Soil characterisation

Property	Value
pH	
Water	5.21 ± 0.02
0.01 M CaCl$_2$	4.84 ± 0.02
1.0 M KCl	4.40 ± 0.02
Texture (volumetric fractions)[a]	
Coarse sand $r > 500$ mm	10%
Fine sand 500 mm $> r > 62$ mm	25%
Silt+clay $r < 62$ mm	65%
Organic matter fraction (w/w)	10.9%
CEC	9.3 cmol kg^{-1}
Anions studied	mg anion/kg soil
Cl$^-$	55
NO$_3^-$	432
PO$_4^{3-}$	< 1
SO$_4^{2-}$	69
Metals studied	mg metal/kg soil
Fe	15×10^3
Mn	480
Cr	60
Zn	68
Cu	37
Pb	< 10

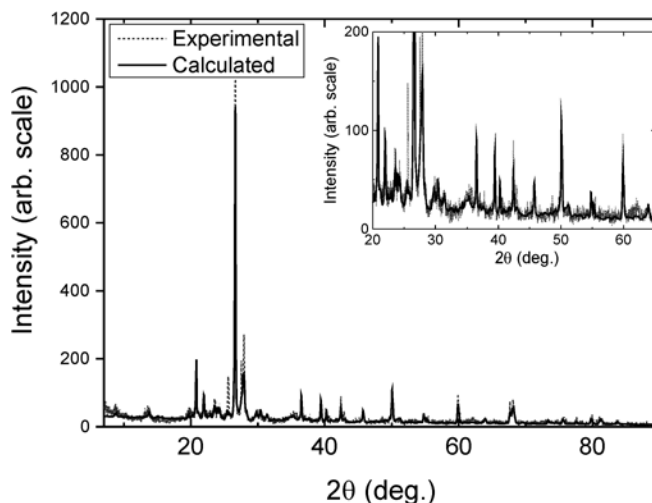[a]*r* is the particle size (in the larger direction).

Figure 1 X-ray diffraction pattern of the mineral fraction below 62 μm, both experimental and fitted with the Rita-Rista method.

Pb(II) interact with carboxylate groups (Orsetti *et al.,* 2006; Zhang *et al.,* 2009). Other spectra features are typical of soil systems.

Pb(II) speciation experiments

Time evolution

Figure 4 shows the results of the Pb distribution among soil fractions for summer and winter conditions expressed as percent of overall metal contents. The time evolution is shown by linear regression lines.
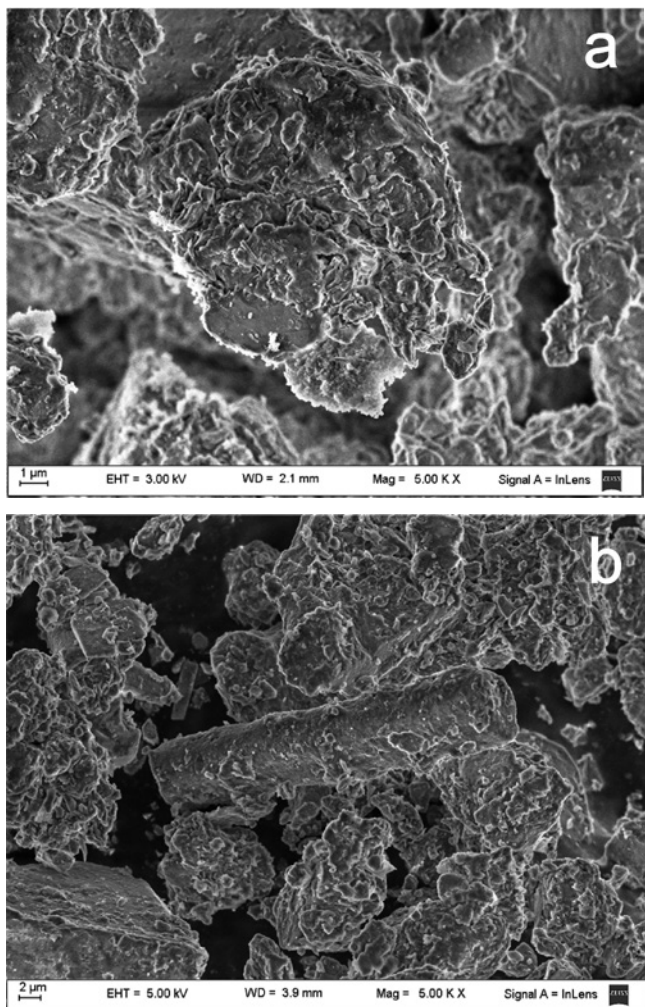
Figure 2 SEM characterisation of the soil under study: SEM images of (a) pristine and (b) polluted soil.
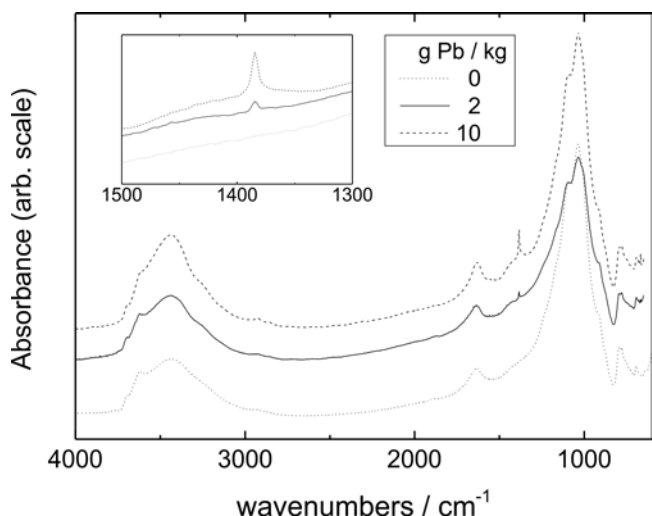

Figure 3 FTIR spectra of pristine soil sample, and the same soil containing increasing amounts of Pb(II), specified as g Pb per kg of soil. Inset: detail of the 1300–1500 cm^{-1} region: the increase of the carboxylate band at 1384 cm^{-1} is clear.
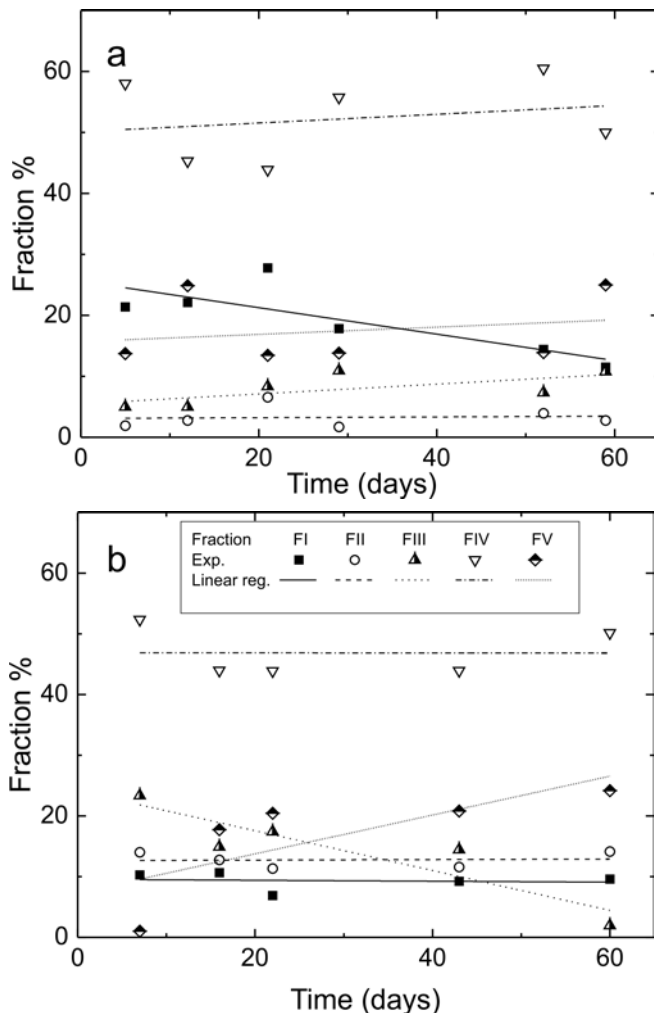

Figure 4 Pb speciation between soil fractions as defined in the selective sequential extraction procedure, 200 mg kg^{-1} total Pb content: (a) summer; (b) winter. Lines are linear regressions.

In the summer experiment (Figure 4a), fraction I shows a decrease whereas all the others present a slight increase; fraction IV is the more important, retaining about 50–60% of total Pb. Fraction I is the second most important at the start, but it decays over time, whereas fractions III, IV and V increase slightly; fraction II remains constant during this experiment. At the end of the run, fraction V becomes the second most important, reaching about 20% of total Pb.

In the winter (Figure 4b), fraction V increases whereas fraction III decreases, compensating each other, the rest being constant. Fraction IV is the most important also in this experiment (about 50% of all Pb), whereas fractions I and II are almost constant in time, each one retaining about 10–15%.

Clearly, comparing the two experiments it is found that there are differences between the two climate conditions (around 10 ºC), which should be related to the differences in ambient conditions. Table 3 presents the minimum, maximum and mean temperatures registered during both experiments; it was observed that the soil remained wet in winter, whereas in summer the soil was dry most of the time.

The different behaviour of both assays can be rationalised considering the environment conditions: the rainfall and plots

Table 3 Temperatures during the experimental periods

Season	Month	Temperature (°C)		
		Lowest	Mean	Highest
Winter	August	1.1	11.2	23.1
	September	4.0	15.9	28.8
	December	10.6	22.6	33.7
Summer	January	14.2	25.7	38.8
	February	13.6	24.5	35.4

were subjected to the same irrigation regime, air temperature being the main difference between the seasons. This means there was a different evaporation regime and as a consequence, the predominant conditions were dry during the whole summer experiment (including periods of complete drought) and wet during the winter time. Thus, the time evolution of speciation should be considerably slower during the summer experiment compared with the winter one because all the chemical processes become slower and even stopped in the absence of water. On the other hand, local winter conditions were not cold enough to freeze the soil water. Consequently, the changes in the last experiment are expected to take place faster than in the first one. In the summer, fraction I decreases over time in the interval studied: this fraction is the most labile and clearly it is the initial form of Pb in most cases when it is incorporated in soil (Orroño and Lavado, 2009). It is observed in Figure 4a that fraction I decreases whereas almost all others increase, indicating progressive incorporation of the metal ions in more stable species. In the winter experiment (Figure 4b), the time evolution is faster, the stage shown in Figure 4a being expected to have already taken place. This is supported by the behaviour of fraction I, which is in Figure 4b lower (less than 10%) than in the summer essay and almost constant; in the winter experiment the main variations are found in fractions III (decreasing) and V (increasing), again showing the evolution towards more stable (mineral) forms.
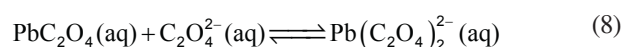
Chemical speciation

Fraction I, the soluble/exchangeable part, is usually considered to be the bioavailable one, albeit in particular cases other species can contribute to bioavailability (Lavado *et al.*, 2004; Luthy *et al.*, 2003). The results shown in Figure 4 indicate that this fraction decreases with time after Pb incorporation in soil, reaching a constant level of about 10%, which is to be expected as the contaminant evolves to more stable species.

Regarding fraction II, a noticeable fact is the difference observed in fraction II when comparing both experiments, being higher in the winter; also, it can be seen that the sum of fractions II plus IV is nearly equal in both experiments. The reactant of step II contains 1 M ammonium acetate, an the acetate ion can coordinate Pb^{2+} ions, albeit less strongly than oxalate, thus it can remove weakly adsorbed Pb(II) species. The difference between summer and winter results for fraction II can be attributed to the fact that, in the former case, the drier

conditions favour, once adsorbed, the evolution of weakly to more strongly adsorbed forms via water removal, thus leaving in summer less Pb(II) available to acetate complexation.

The behaviour of fraction III is interesting, showing an increase in the summer experiment and a decrease in the winter one; as discussed above, the former case corresponds to an earlier stage in the evolution of Pb in soil, thus indicating that the organic bound metal is a transient stage between labile forms and more stable mineral forms.

Fraction IV shows the higher Pb retention. This fraction has been described (Ma and Uren, 1998) as associated to iron and aluminium oxides; however, in the present case very little, if any, of these minerals are found. The oxalate ion has strong complexing action, enabling removal of adsorbed metal species. The reactions involved here are:

$$Pb^{2+}(aq) + C_2O_4^{2-}(aq) \rightleftharpoons PbC_2O_4(aq) \tag{7}$$

$$PbC_2O_4(aq) + C_2O_4^{2-}(aq) \rightleftharpoons Pb(C_2O_4)_2^{2-}(aq) \tag{8}$$

As in fraction I, the precipitation of lead oxalate could, in principle, arise:

$$PbC_2O_4(aq) \rightleftharpoons PbC_2O_4(s) \tag{9}$$
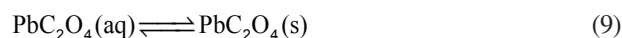
An estimation of the Pb(II) speciation, employing the Visual Minteq program, according to reactions (7)–(9) in the presence of the step IV reactants (oxalic acid and ammonium oxalate) shows that saturation of $PbC_2O_4(s)$ is reached at a total Pb concentration of about 0.8 mM, consequently no precipitation should occur in the experimental conditions. Furthermore, because about half of total Pb (100 mg kg^{-1} Pb in soil) is found in fraction IV, a total Pb(II) concentration of ~ 5 × 10^{-6} M should result by the action of the oxalate extractant; the free Pb^{2+} equilibrium concentration in these conditions is found to be approximately $c = 1.9 \times 10^{-8}$ M. If lead is adsorbed onto feldspars (or other aluminosilicates), it is expected that under these conditions it would be essentially removed. The main mineral phases are feldspars and quartz; both mineral types are known to have a low adsorption capacity, quartz having the lower one (Wu *et al.*, 2003). Adsorption studies of lead (and heavy metals, in general) on feldspars are scarce. We will analyse here some aluminosilicate minerals in order to estimate a limit to the remaining amount of Pb adsorbed in fraction IV. In a study of metal adsorption onto several soil minerals, with particle size and properties consistent with a silt fraction (Frimmel and Huber, 1996), the adsorption of Pb onto quartz, orthoclase and albite (among other minerals) was interpreted with the Freundlich isotherm:

$$q = Kc^n \tag{10}$$

where q is the amount of Pb adsorbed, K the adsorption coefficient and n an empiric parameter; from the fitted parameters (at pH = 8.4) it is found that the amount of Pb adsorbed in the presence of the step IV oxalate reactant is: for quartz ($K = 0.14$ mmol kg^{-1}, $n = 1.8$) $q \approx 0.01$ ppm; for orthoclase ($K = 0.17$ mmol kg^{-1}, $n = 1.2$) $q \approx 0.11$ ppm and for albite ($K = 0.27$ mmol kg^{-1}, $n = 1.1$) $q \approx 0.26$ ppm. In another study, Sepehrian *et al.* (2011) investigated the adsorption of lead onto mesoporous aluminosilicates (with a surface area A

= 834 m^2 g^{-1}) interpreting the results in terms of a simple Henry isotherm [Eqn (10) with $n = 1$]; of the several aluminosilicates studied, the stronger adsorbent had $K = 763$ L kg^{-1}; for the c value found above, that predicts an amount $q = 1.4 \times 10^{-6}$ mol kg$^{-1} \approx 0.1$ ppm. These considerations support the assumption that Pb(II) will be mostly desorbed in the presence of the oxalate/oxalic acid reactant. The high proportion of Pb(II) found in fraction IV here can consequently be attributed to adsorption onto quartz and aluminosilicate materials, which constitute the major part of the soil minerals, as revealed by the DRX and SEM observations (Figures 1 and 2, respectively); adsorption to the amorphous materials is also to be expected.

The Pb(II) speciation among the different (operationally defined) soil fractions shows that, in the timeframe studied, Pb(II) recently added to the soil is mostly adsorbed onto the mineral fraction, with smaller quantities found in the soluble/exchangeable form, associated to reducible forms, fixed into the mineral fraction (fraction V, the most stable form) and associated to organic matter; these two last fractions varying complementarily with time in favour of fraction V. In fact, it is conceivable that part of the metal proportion found in fraction IV could become displaced from previous fractions in the course of the SSE, by adsorption of part of the Pb(II) released in steps II and III; unfortunately, at present there are no available data to analyse this point. Nevertheless, the importance of adsorbed species in the state of Pb(II) in soil is clearly stated. Comparing the two experiments, it is seen that in the summer fraction I decays with time whereas in the winter it has reached a relatively low level, thus we conclude that the summer experiment has a slower evolution, and the results of Figure 4a correspond to an earlier stage of the Figure 4b experiment. Also, in Figure 4 a fractions III, IV and V show a slow increase which is reasonable for an early stage in the Pb(II) speciation evolution.

Thus, most Pb is found to be in relatively stable forms, expected to be non bioavailable and evolving towards more inert species. Similar behaviour for Pb in soils was found in other studies (Cajuste *et al.,* 2000; Shrivastava and Banerjee, 2004; Zhao *et al.,* 2009; Favas *et al.,* 2011; Kabala *et al.,* 2011).

CONCLUSIONS

The results of the present study show that the speciation and the interaction with different soil phases of Pb(II) is dependent on climate conditions, at least when the metal has been recently incorporated in soil. In the studied range, soil water content would be more important than temperature. The results also strongly suggest that, even at a short time after the pollutant addition only a relatively low fraction of Pb in the studied soil is bioavailable. The most important part of Pb(II) is bound by adsorption to the mineral fraction. However, the most stable (mineral incorporated) form is observed to increase with time, which suggests that, in the long term, a higher proportion of Pb(II) would evolve in this way, as has been reported in the literature.

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